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Using the Lambert Function to Map Enzyme Kinetics

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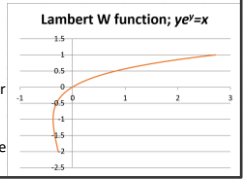
ABSTRACT

In an academic setting, enzyme kinetics are introduced by analysis of the initial rates using the Michaelis-Menten model. To better understand enzyme kinetics, a complete time course analysis will provide a larger set of information. The use of the integrated form of the rate equation allows for a beginning glance at the mechanism of reaction. With this, the Lambert W function can be used to analyze the integrated rate equations. There are two different branches of the Lambert W function, a negative branch and a positive branch. For each branch there is a unique expanded series approximation used for analysis of data. This experiment will demonstrate how to use the integrated rate equations to determine which branch of the Lambert W function is best for analysis of a particular enzyme. Analysis of wheat germ acid phosphatase acting on p-nitrophenyl phosphate will be completed to show an example of how this application can be used.

BACKGROUND

$$t = -\frac{1}{V^{app}}(K^{app}\ln\frac{[S_t]}{[S_o]} + P_t)$$

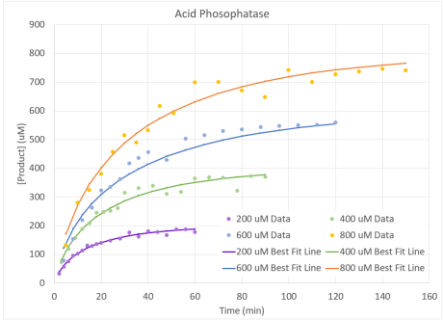
During an enzyme catalyzed reaction, the product accumulates over the time of the reaction. To study the reaction, the initial rate equation, also know as the Michalis-Menten model is used. The Lambert W function will also be used in this experiment. This function makes an algebraic approximation for the equation **x=ye^y** . This approximation allows us to take the open form of time used in the integrated version of the rate equation and transform it into a closed form. Since the Lambert function is an approximation of an exponential function, there are different approximations for each arm of the function. As seen on the left, there is both a negative and positive arm, each with its own approximation. While these approximations each extend across the origin, the relative percent error increases, therefore decreasing the effectiveness of the transformation. Since this reaction is very sensitive to each moving part, we want to limit the amount of error wherever possible.



FINDING THE MOLAR ABSORPTIVITY

To determine an accurate measure of the molar absorptivity of the p-nitrophenol, solutions were made with varying concentrations of Na_2CO_3 and a constant concentration of the product. The absorbance of these solutions was measured and Beer's Law used to find the molar absorptivity at each salt concentration. For this project, the molar absorptivity of p-nitrophenol was determined to be 18500 M^{-1} cm^{-1}. It was also shows that there is no statistical difference between the molar absorptivities at different salt concentrations. This allows us to assume a standard constant for each reaction here on out. The concentration of the substrate was determined from the published molar absorptivity and the measurement of the absorbance at 312 nm.

FINDING THE V^{APP} AND K^{APP}

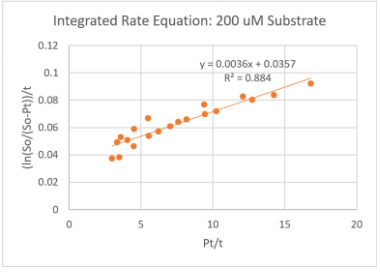


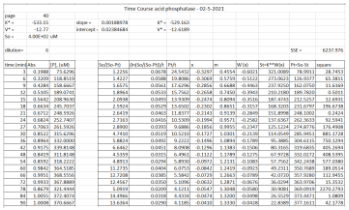
1. Calculating the Product

To calculate the amount of product at various times during the reaction, aliquots were taken from the reaction mixture and stopped using a 1 M Na_2CO_3 solution, in operando. The absorbance at 405 nm was measured for each sample and Beer's Law was used to find the amount of product at each time. This reaction was repeated with various amounts of substrate. The graph to the left shows this reaction.

2. Integrated Rate Equation

To determine which version of the Lambert W function to use, the data was graphed using the Integrated Rate Equation. If the graph returned has a positive slope, the approximation for the negative branch of the Lambert function should be used. If the graph returned has a negative slop, the approximation for the positive branch of the Lambert function should be used. The graph to the left shows the measured reaction, an example of a graph with a positive slope, and therefor product inhibition.





3. Minimizing the Sum of the Square

To determine the best fit V^{app} and K^{app}, Excel was used. First, the Lambert W function was used to find the predicted product concentration at each time mark. The Solver function of Excel was used to minimize the difference between the actual amount and predicted amount of product. This was done by changing the prediction values of the K^{app} and V^{app}. This process was also done by hand as the Solver program can get stuck in local minima while searching for the global minima. The product concentration found by the Lambert W function was then plotted next to the experimental concentration (see graph from step 1).

$$\frac{\ln\frac{S_o}{S_t}}{t} = \frac{V^{app}}{K^{app}} - \frac{1}{K^{app}} * \frac{P_t}{t}$$

4. Finding the K_i, V_{max}, and K_m

Once the SSE has been minimized, the K_i, V_{max}, and K_m can be found. The V^{app} has both the K_i and the K_m incorporated into it. The K_i is the dissociation constant for the EP product. To solve for the K_i and the K_m, the equation on the left is used. Since the collective K^{app} value incorporates both constants, the same math can be applied to reactions run using an added inhibitor. This process can also be used to find a global V_{max} and K_m.

$$K^{app} = \frac{K_m(K_i + S_o)}{(K_i - K_m)}$$

SUMMARY

To complete a reaction in this convenient analysis method, the steps below should be followed:

1. Run the enzyme reaction time course and record the absorbances.
2. Plot the reaction using the integrated rate equation.
3. Use the slope to determine which branch of the Lambert W function to use.
    a. Positive slope: negative branch
    b. Negative slope: positive branch
4. Fit the data using the corresponding approximation of the Lambert W function and minimize the sum of squares using the Solver function of Excel.
5. Determine the K_i, V_{max}, and K_m using the conversion equation.

FUTURE WORK

In the future we will continue to develop methods for conveniently analyzing enzyme reaction time course. We have also started work on incorporating inhibitors and would like to continue this work to show true product inhibition. There are also other substrates and enzymes we wish to test as each reaction could produce different models of inhibition.

REFERENCES

Golicnik, M. (2012). On the Lambert W function and its utility in biochemical kinetics. *Biochemical Engineering Journal*, *63*, 116–123. https://doi.org/10.1016/j.bej.2012.01.010

ACKNOWLEDGEMENTS

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